Fig. 1a

L2 cDNA Library

L2 cDNA library in λ gt10

18,000 plaques

screen ⟶ ³²P-ss cDNA of A 20.2↓

No signal
(814 plaques)

Spot on Bacterial lawn

Duplicate filter

screen ⟶ ³²P-ss cDNA of A 20.2↓

Signal to L2,ss cDNA probe
(114 plaques)

No signal
(372 plaques)

Subtracted ³²P-ss cDNA of L2 ✻

screen

188 clones

insert
(72)

No insert
(300)

cut phage DNA
with EcoRI

screen ⟶ Signal to L2 and A 20.2↓
ss cDNA probes
(128 plaques)

³⁵S-ss cDNA probe of L3

screen

132 inserts on Gene Screen Plus

Hybridization to known sequences † and
elimination of very small inserts

Signal
(61 inserts)

No signal
(71 inserts)

Northern analysis ‡

Not T cell specific

No signal
detected

Specificity assigned

~40% (29 inserts)   (28 inserts)   (14 inserts)
~40% (29/71)   ~40% (28/71)   ~20% (14/71)

Fig. 1b

L3 cDNA Library

L3 cDNA Library in λ gt 10

8,000 plaques

screen ⟶ ³²P-ss cDNA of A 20.2↓

No signal
(150 plaques)

Prepare phage DNA and
transfer to Gene Screen Plus

screen ⟶ ³⁵S-ss cDNA of A 20.2↓

Signal
(33 clones)

No signal
(117 clones)

insert
(56)

No insert
(61)

Hybridization to known sequences †

³⁵S-ss cDNA probe of L2

screen

Signal
(14 inserts)

No signal
(40 inserts)

Northern analysis ‡

Not T cell specific

No signal detected

Specificity assigned

~47% (19 inserts)   (13 inserts)   (8 inserts)
~47% (19/40)   ~32% (13/40)   ~20% (8/40)

No signal
(54 inserts)

Fig. 2A

```
-145                                                                    ATGTC

-140   CATGAACTGC TGAGTGGATA AACAGCACGG GATATCTCTG TCTAAAGGAA TATTACTACA CCAGGAAAAG

-70    GACACATTCG ACAACAGGAA AGGAGCCTGT CACAGAAAAC CACAGTGTCC TGTGCATGTG ACATTTCGCC

  1    ATG GGA AAC AAC TGT TAC AAC GTG GTG GTC ATT GTG CTG CTG CTA GTG GGC TGT GAG AAG   60
  1    Met Gly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu Val Gly Cys Glu Lys   20

 61    GTG GGA GCC GTG CAG AAC TCC TGT GAT AAC TGT CAG CCT GGT ACT TTC TGC AGA AAA TAC   120
 21    Val Gly Ala Val Gln Asn Ser Cya Asp Aan Cya Gln Pro Gly Thr Phe Cys Arg Lys Tyr   40

121    AAT CCA GTC TGC AAG AGC TGC CCT CCA AGT ACC TTC TCC AGC ATA GGT GGA CAG CCG AAC   180
 41    Asn Pro Val Cys Lys Ser Cys Pro Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Aan   60

181    TGT AAC ATC TGC AGA GTG TGT GCA GGC TAT TTC AGG TTC AAG AAG TTT TGC TCC TCT ACC   240
 61    Cys Asn Ile Cys Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lya Phe Cys Ser Ser Thr   80

241    CAC AAC GCG GAG TGT GAG TGC ATT GAA GGA TTC CAT TGC TTG GGG CCA CAG TGC ACC AGA   300
 81    Hia Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro Gln Cys Thr Arg  100

301    TGT GAA AAG GAC TGC AGG CCT GGC CAG GAG CTA ACG AAG CAG GGT TGC AAA ACC TGT AGC   360
101    Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr Lys Gln Gly Cys Lys Thr Cys Ser  120

361    TTC GGA ACA TTT AAT GAC CAG AAC GGT ACT GGC GTC TGT CGA CCC TGG ACG AAC TGC TCT   420
121    Leu Gly Thr Phe Aan Asp Gln Asn Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser  140

421    CTA GAC GGA AGG TCT GTG CTT AAG ACC GGG ACC ACG GAG AAG GAC GTG GTG TGT GGA CCC   480
141    Leu Asp Gly Arg Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro  160

481    CCT GTG GTG AGC TTC TCT CCC AGT ACC ACC ATT TCT GTG ACT CCA GAG GGA GGA CCA GGA   540
161    Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu Gly Gly Pro Gly  180

541    GGG CAC TCC TTG CAG GTC CTT ACC TTG TTC CTG GCG CTG ACA TCG GCT TTG CTG CTG GCC   600
181    Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu Ala Leu Thr Ser Ala Leu Leu Leu Ala  200

601    CTG ATC TTC ATT ACT CTC CTG TTC TCT GTG CTC AAA TGG ATC AGG AAA AAA TTC CCC CAC   660
201    Leu Ile Phe Ile Thr Leu Leu Phe Ser Val Leu Lva Trp Ile Arg Lys Lys Phe Pro His  220

661    ATA TTC AAG CAA CCA TTT AAG AAG ACC ACT GGA GCA GCT CAA GAG GAA GAT GCT TGT AGC   720
221    Ile Phe Lys Gln Pro Phe Lys Lys Thr Thr Gly Ala Ala Gln Glu Glu Asp Ala Cys Ser  240

721    TGC CGA TGT CCA CAG GAA GAA GAA GGA GGA GGA GGA GGC TAT GAG CTG TGA TGTACTATC   780
241    Cys Arg Cys Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Tyr Glu Leu ---
```
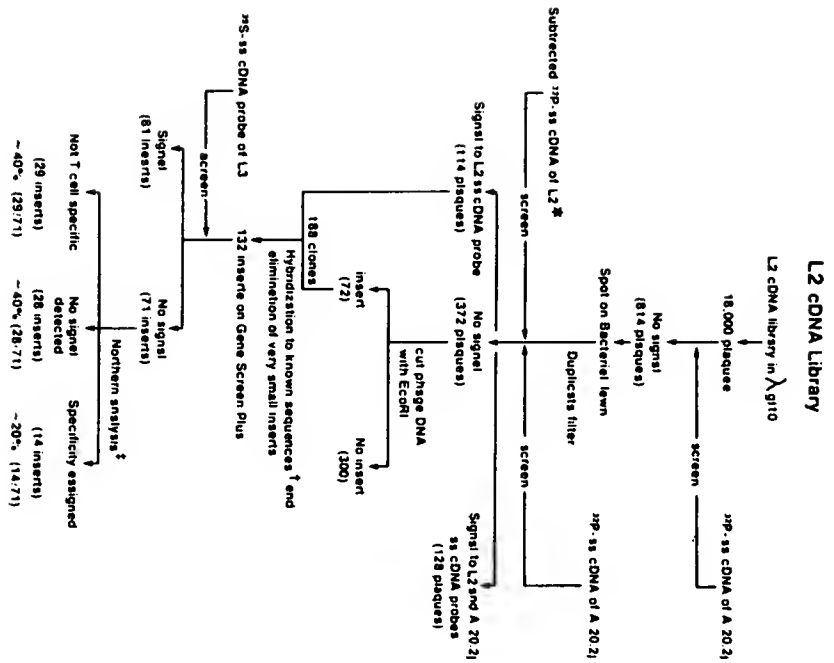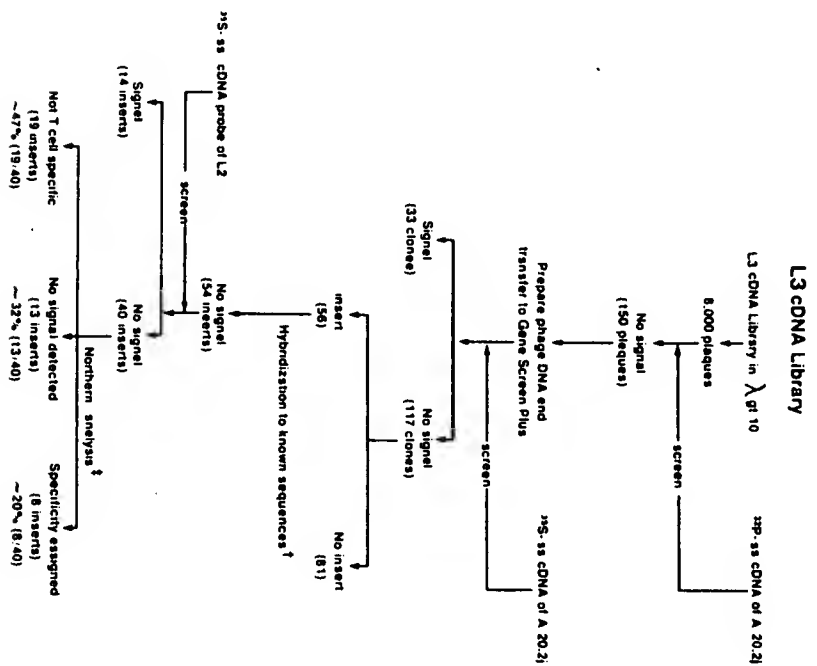
Fig. 2B

```
 781   CTAGGAGATG TGTGGGCCGA AACCGAGAAG CACTAGGACC CCACCATCCT GTGGAACAGC ACAAGCAACC    850

 851   CCACCACCCT GTTCTTACAC ATCATCCTAG ATGATGTGTG GGCGCGCACC TCATCCAAGT CTCTTCTAAC    920

 921   GCTAACATAT TTGTCTTTAC CTTTTTTAAA TCTTTTTTTA AATTTAAATT TTATGTGTGT GAGTGTTTTG    990

 991   CCTGCCTGTA TGCACACGTG TGTGTGTGTG TGTGTGTGAC ACTCCTGATG CCTGAGGAGG TCAGAAGAGA   1060

1061   AAGGGTTGGT TCCATAAGAA CTGGAGTTAT GGATGGCTGT GAGCCGGnnn GATAGGTCGG GACGGAGACC   1130

1131   TGTCTTCTTA TTTTAACGTG ACTGTATAAT AAAAAAAAAA TGATATTTCG GGAATTGTAG AGATTGTCCT   1200

1201   GACACCCTTC TAGTTAATGA TCTAAGAGGA ATTGTTGATA CGTAGTATAC TGTATATGTG TATGTATATG   1270

1271   TATATGTATA TATAAGACTC TTTTACTGTC AAAGTCAACC TAGAGTGTCT GGTTACCAGG TCAATTTTAT   1340

1341   TGGACATTTT ACGTCACACA CACACACACA CACACACACA CACGTTTATA CTACGTACTGT TATCGGTAT   1410

1411   TCTACGTCAT ATAATGGGAT AGGGTAAAAG GAAACCAAAG AGTGAGTGAT ATTATTGTGGA GGTGACAGA   1480

1481   CTACCCCTTC TGGGTACGTA GGGACAGACC TCCTTCGGAC TGTCTAAAAC TCCCCTTAGA AGTCTCGTCA   1550

1551   AGTTCCCGGA CGAAGAGGAC AGAGGAGACA CAGTCCGAAA AGTTATTTTT CCGGCAAATC CTTTCCCTGT   1620

1621   TTCGTGACAC TCCACCCCTT GTGGACACTT GAGTGTCATC CTTGCGCCGG AAGGTCAGGT GGTACCCGTC   1690

1691   TGTAGGGGCG GGGAGACAGA GCCGCGGGGG AGCTACGAGA ATCGACTCAC AGGGCGCCCC GGGCTTCGCA   1760

1761   AATGAAACTT TTTTAATCTC ACAAGTTTCG TCCGGGCTCG GCGGACCTAT GGCGTCGATC CTTATTACCT   1830

1831   TATCCTGGCG CCAAGATAAA ACAACCAAAA GCCTTGACTC CGGTACTAAT TCTCCCTGCC GGCCCCCGTA   1900

1901   AGCATAACGC GGCGATCTCC ACTTTAAGAA CCTGGCCGCG TTCTGCCTGG TCTCGCTTTC GTAAACGGTT   1970

1971   CTTACAAAAG TAATTAGTTC TTGCTTTCAG CCTCCAAGCT TCTGCTAGTC TATGGCAGCA TCAAGGCTGG   2040

2041   TATTTGCTAC GGCTGACCGC TACGCCGCCG CAATAAGGGT ACTGGGCGGC CCGTCGAAGG CCCTTTGGTT   2110

2111   TCAGAAACCC AAGGCCCCCC TCATACCAAC GTTTCGACTT TGATTCTTGC CGGTACGTGG TGGTGGGTGC   2180

2181   CTTAGCTCTT TCTCGATAGT TAG AC
```

Fig. 3a

L3G25*4 →
18S —

28S —

K46
EL4 TPA
L3
L3 ConA
LGL

Fig. 3b

L3G14*2 →
18S —

28S —

K46
EL4 TPA
L3
L3 ConA
LGL

Fig. 3c

L3G20*3

18S —

28S —

K46
EL4 TPA
L3
L3 Con A
LGL

Fig. 4
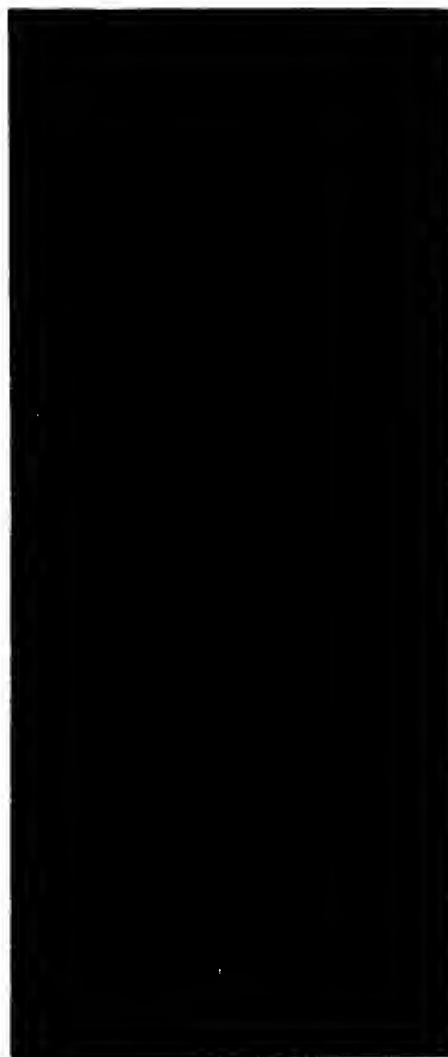
a Fig. 6a

Hrs after Stim.   0 ½ 6 12 24 6
TCR   IL-2

28S-

18S-

b Fig. 6b

Hrs after Stim.   0 ½ 6 12 24 6
TCR   IL-2

28S-

18S-

c Fig. 6c

Hrs after Stim.   0 ½ 6 12 24 6
TCR   IL-2

28S-

18S-

d Fig. 6d

Hrs after Stim.   0 ½ 6 12 24 6
TCR   IL-2

28S-

18S-

Fig. 5a

a

18S —
28S —

K 46
EL 4
EL 4 TPA
L 2
L 2 ConA
L 3
L 3 ConA
LGL

fig. 5b

b

18S —
28S —

K 46
EL 4
EL 4 TPA
L 2
L 2 ConA
L 3
L 3 ConA
LGL

Fig. 7a

Fig. 7b

Fig. 7c

a

28S—
18S—
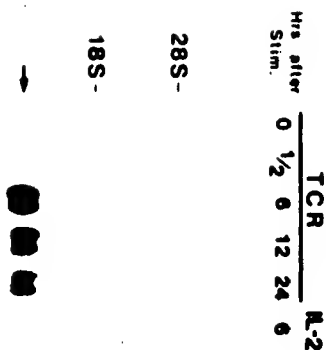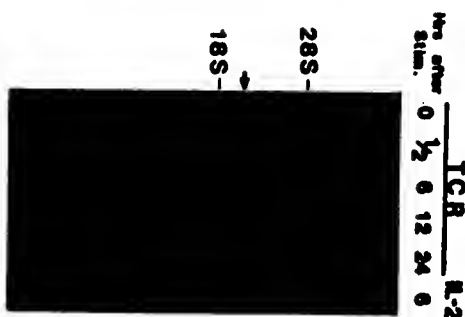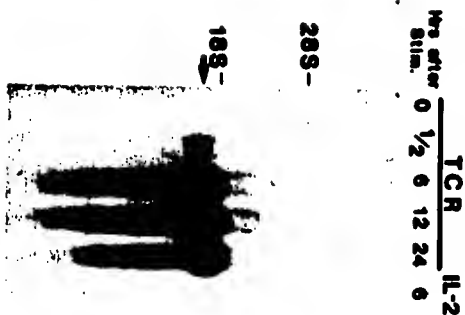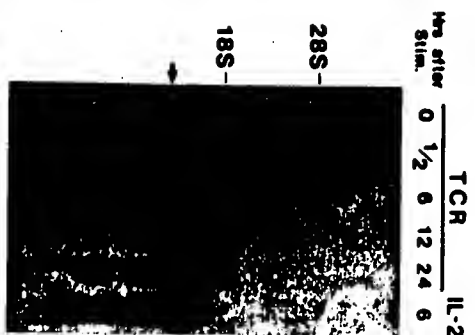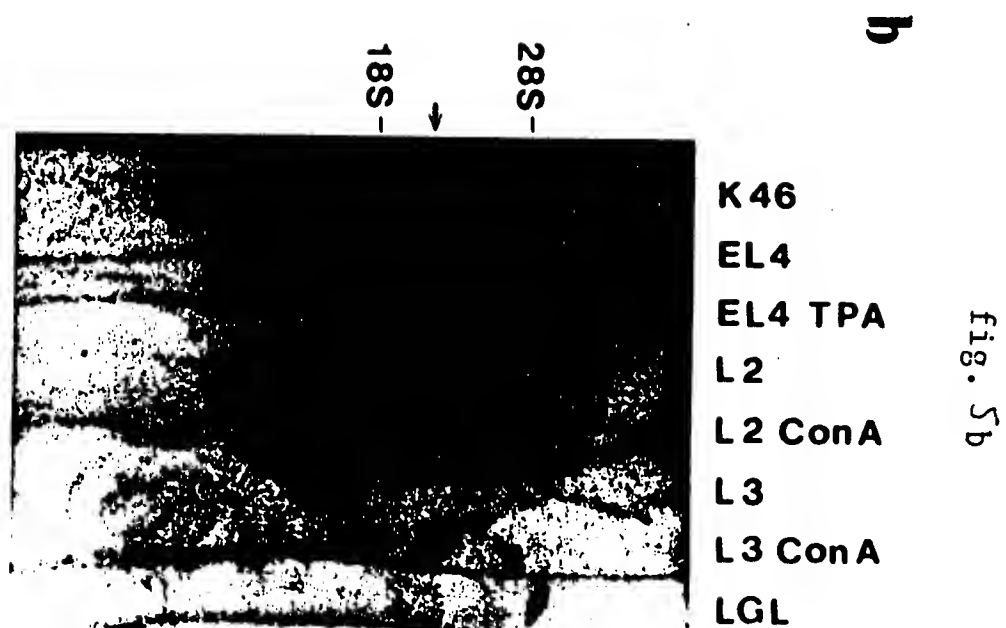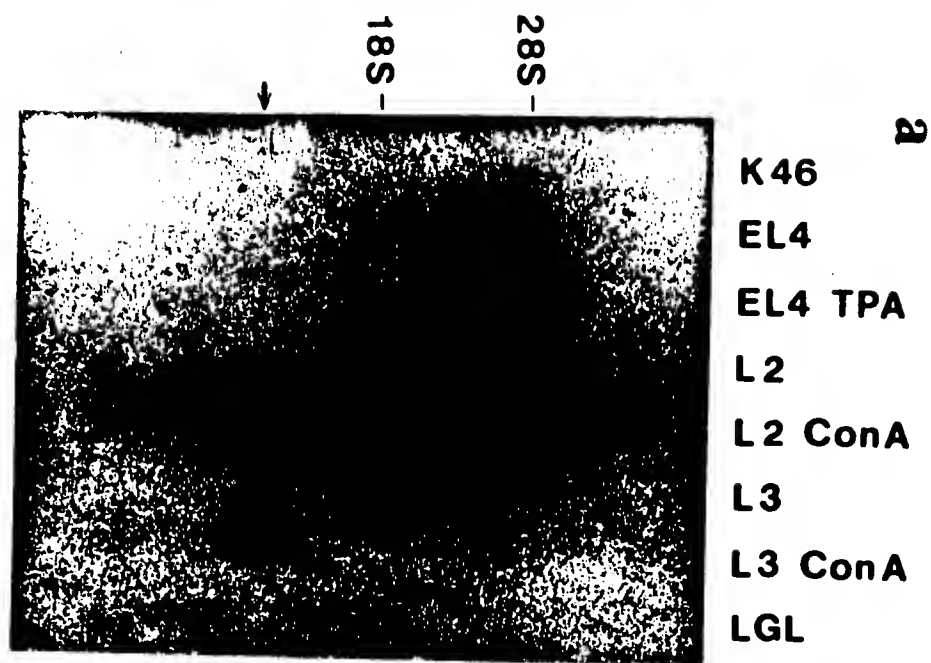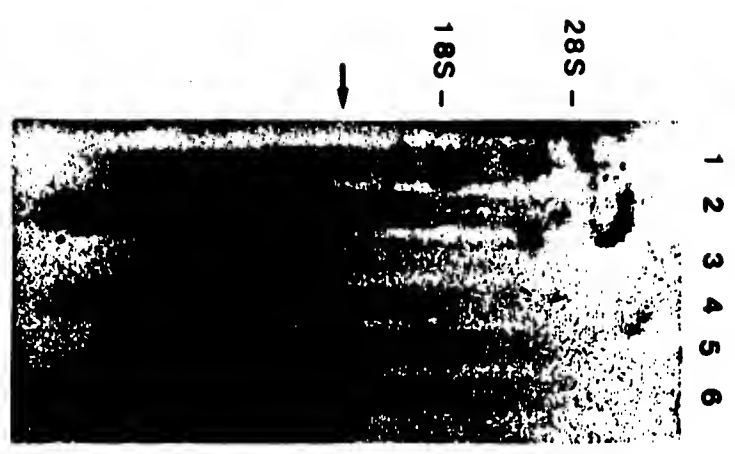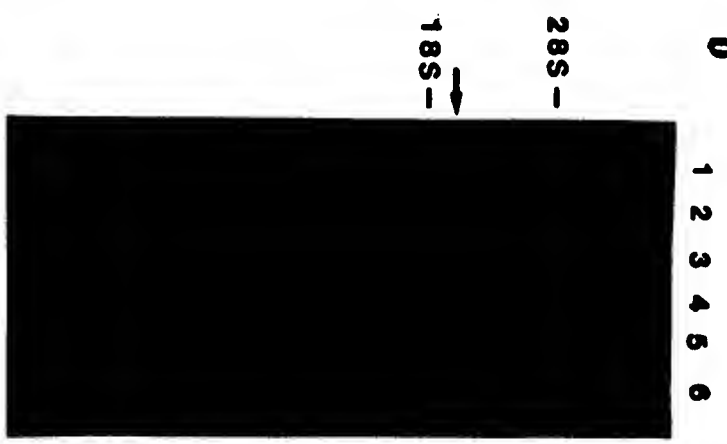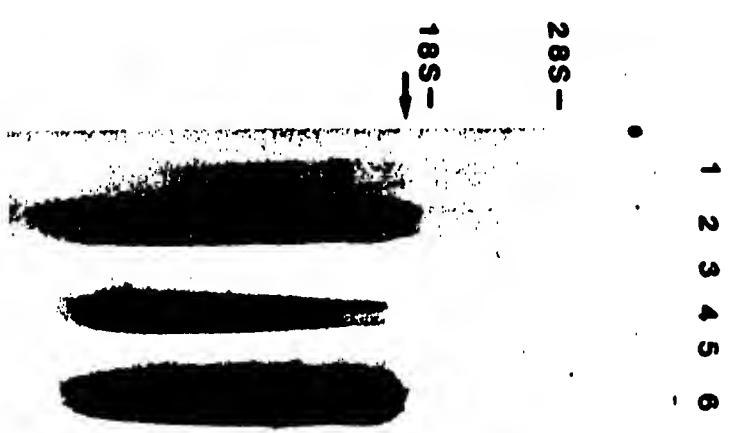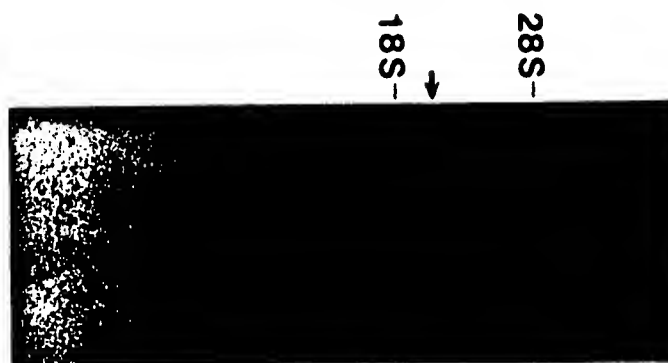
BW 5147
BW 5147 ConA
Md 90
Md 90 ConA
PN 37
PN 37 ConA

Fig. 8a

b

28S—
18S—

L 2
A 11
L 3
Melanocyte
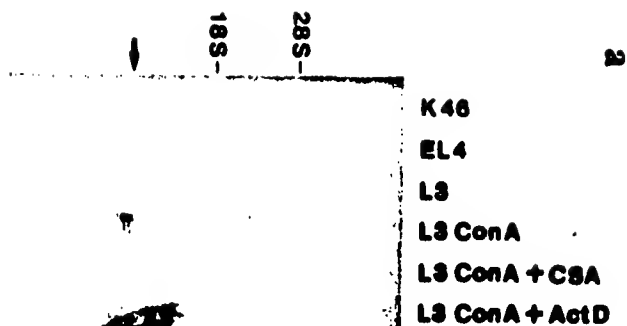
Fig. 8b
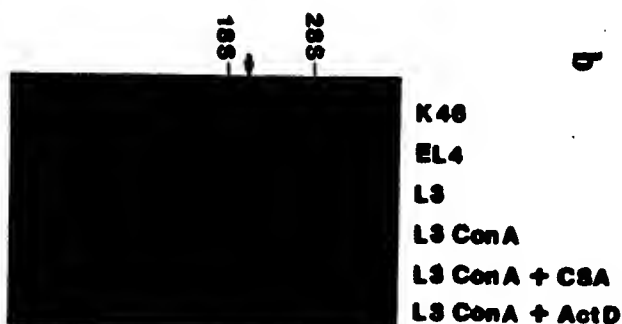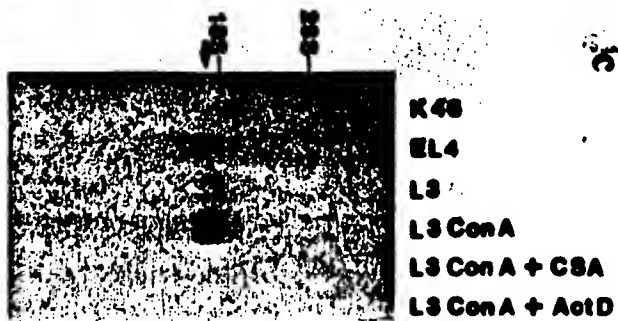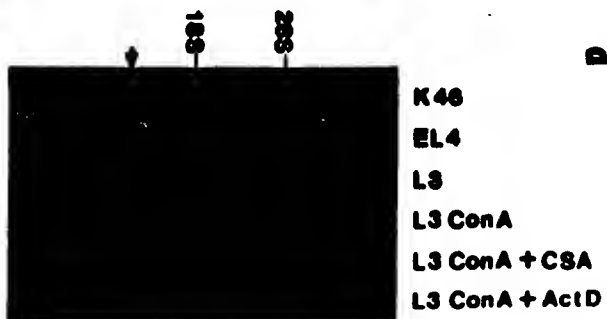
Fig. 9 a

Fig. 9 b

Fig. 9 c

Fig. 9 d

a

28S —

18S —

Fig. 10 a

1 2 3 4

b

28S—

18S—

Fig. 10 b

1 2 3 4

c

28S—

18S—

Fig. 10 c

1 2 3 4

A B C

28S —

18S —

figure 11

A B C D

40kD

Figure 12

A   B   C   D

23 kD ➤

Figure 13

Figure 14

A

CELL NUMBER

300

0

10^0 10^1 10^2 10^3 10^4

INTENSITY

B

CELL NUMBER

300

0

10^0 10^1 10^2 10^3 10^4

INTENSITY

C

CELL NUMBER

300

0

10^0 10^1 10^2 10^3 10^4

INTENSITY

←28S

←18S
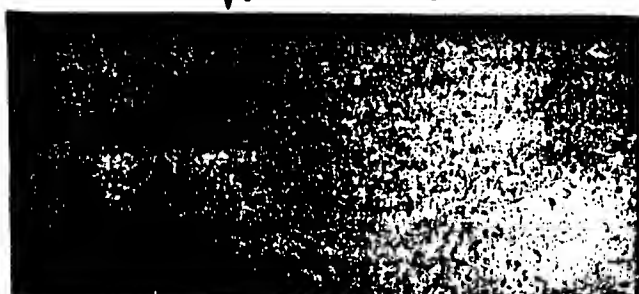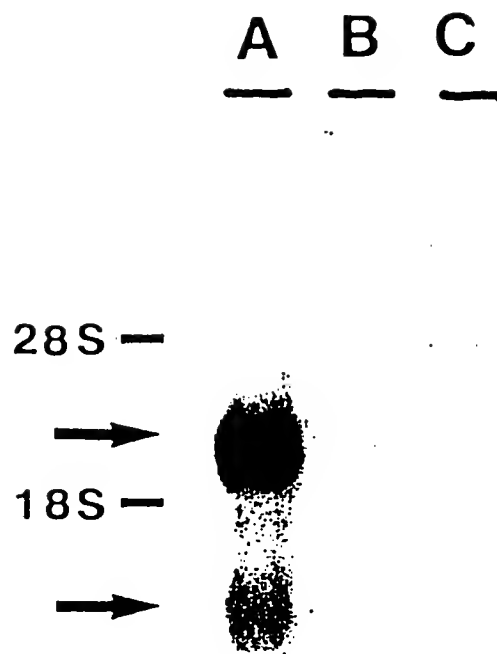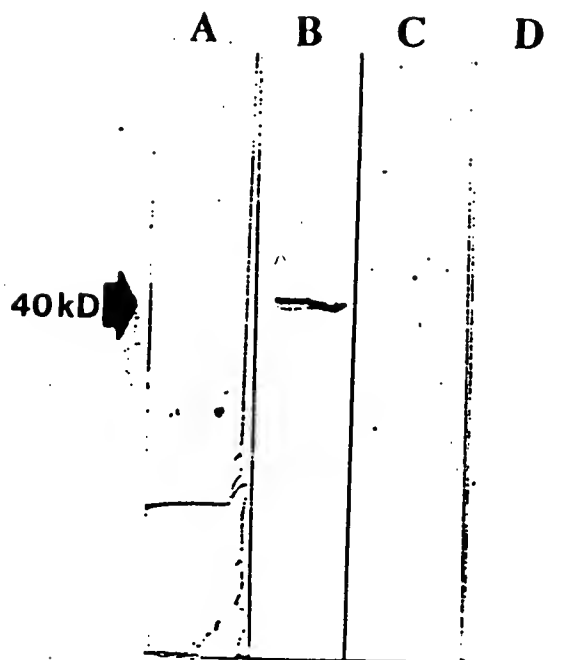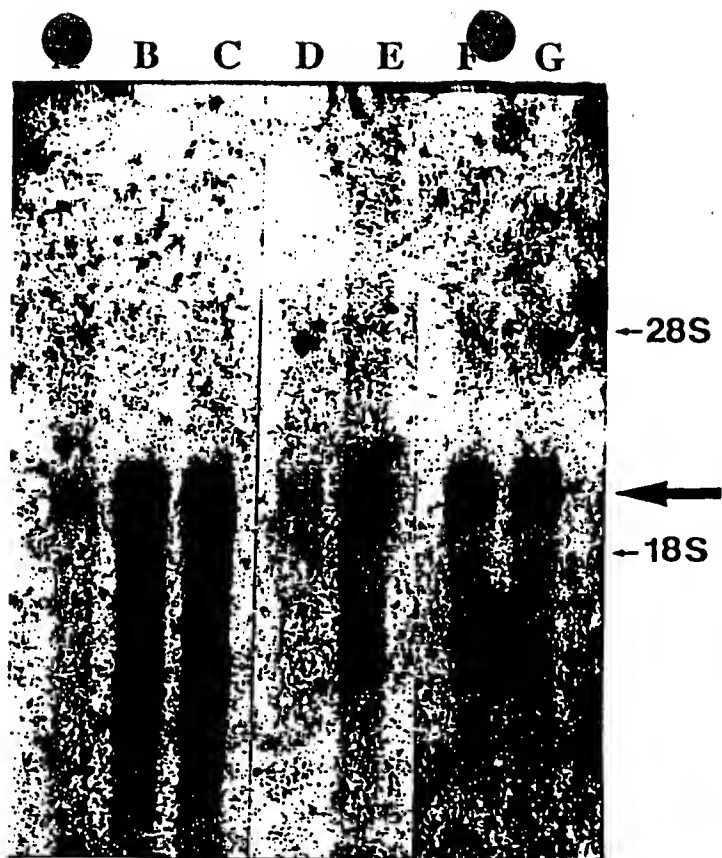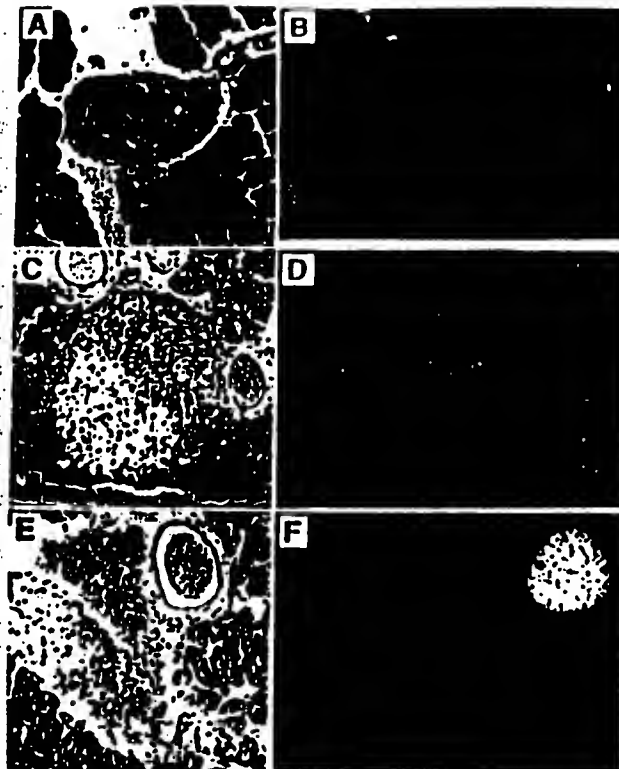
Figure 15

Figure 16

Figure 17

4-1BB	(64)	C R V C A G Y F R F K K - - F - C S S T H N A E C - E C
Sina	(71)	C P V C F D Y V - - - - - I L Q C S S G H L V - C V S C
DG17	(25)	C P I C F E F I - Y K K Q I Y Q C K S G H H A - C K E C

Fig. 18

A B C D E F G H I

28S—

18S—

I →

II →

Fig. 19.

Fig. 20

Figure 21

1 2 M

—32.5
—27.5
rs-4-1BBP

M 1 2 3 4

Figure 22

106.0—
80.0—

49.5—
4-1BB dimer

32.5—
27.5—
4-1BB monomer

18.5—

1 2 3 4

Figure 23

—110

55

30

Figure 24

Hours— 0 3 6 12 18 24 36 48 72 96
A.

—28S

—18S

1 2 3 4 5 6 7 8 9 10

B.

—28S

—18S

1 2 3 4 5 6 7 8 9 10

Figure 25

A.

—28S

—18S

B.

—18S

1 2 3 4 5

Figure 26

A.

—28S

—18S

B.

—18S

1 2 3 4

Figure 27

A.

COUNT

INTENSITY

B.

INTENSITY

C.

200

COUNT

10⁰ 10¹ 10² 10³ 10⁴

INTENSITY

D.

400

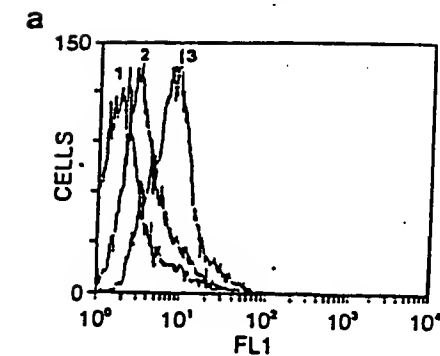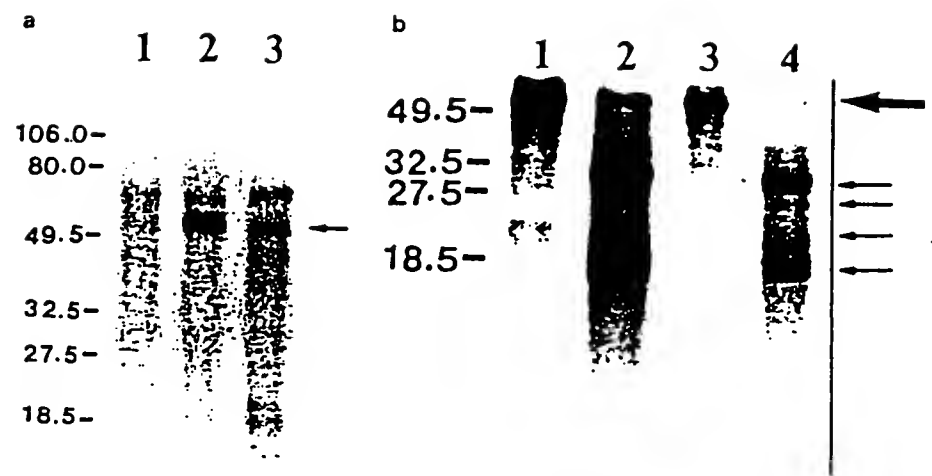10⁰ 10¹ 10² 10³ 10⁴

INTENSITY

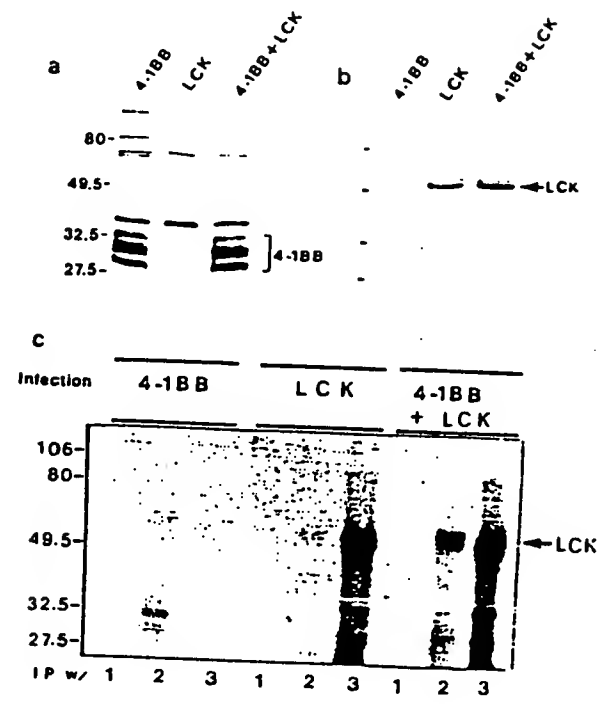Figure 28

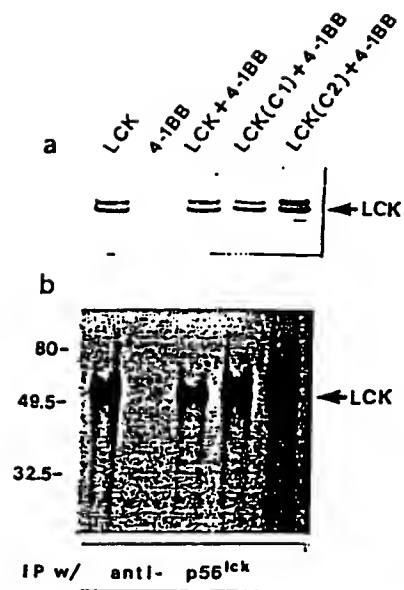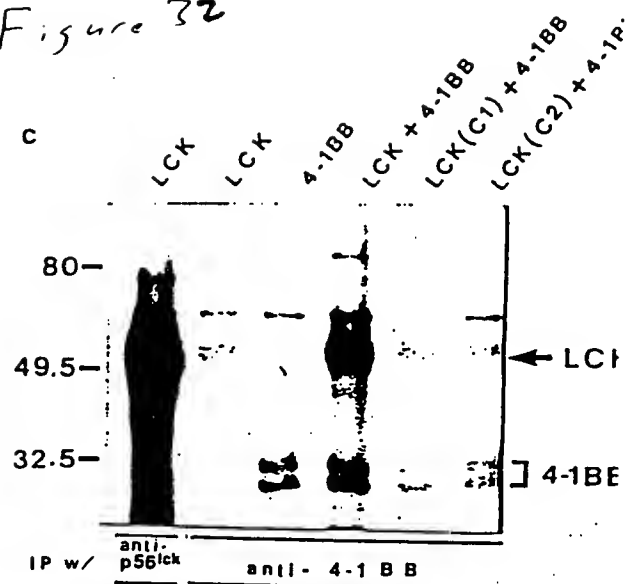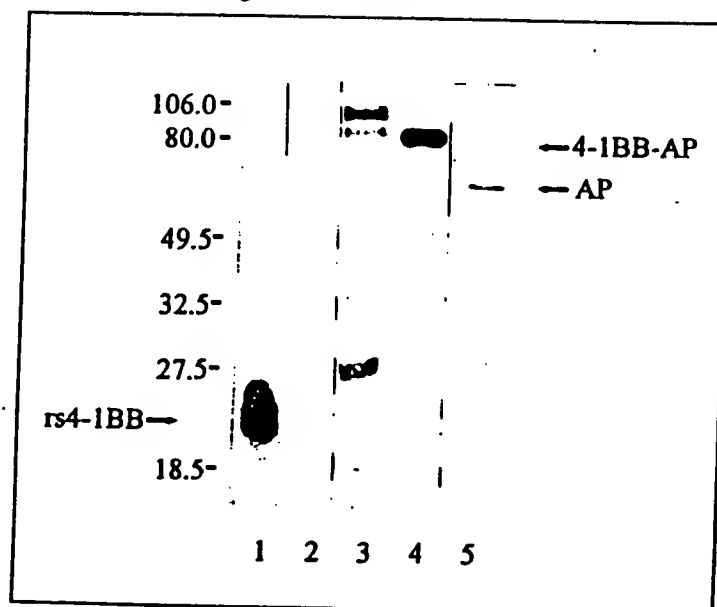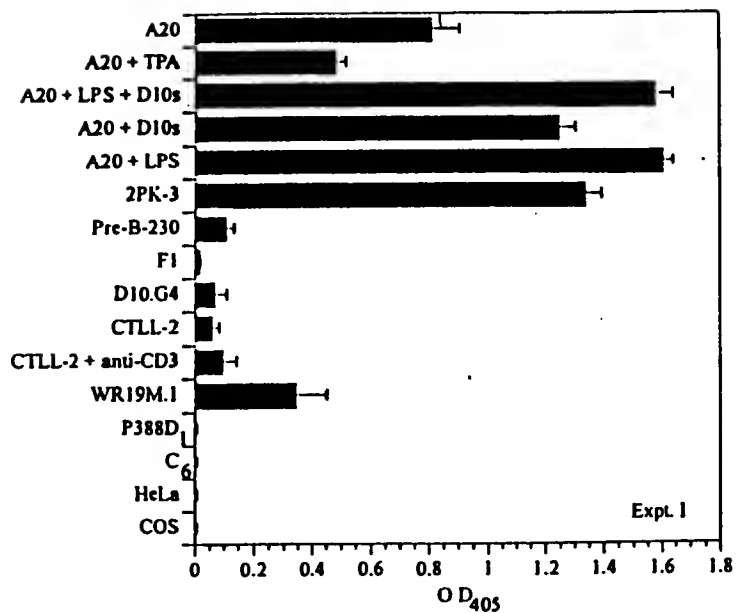Fold increase-   5.6x   6.9x   4.1x
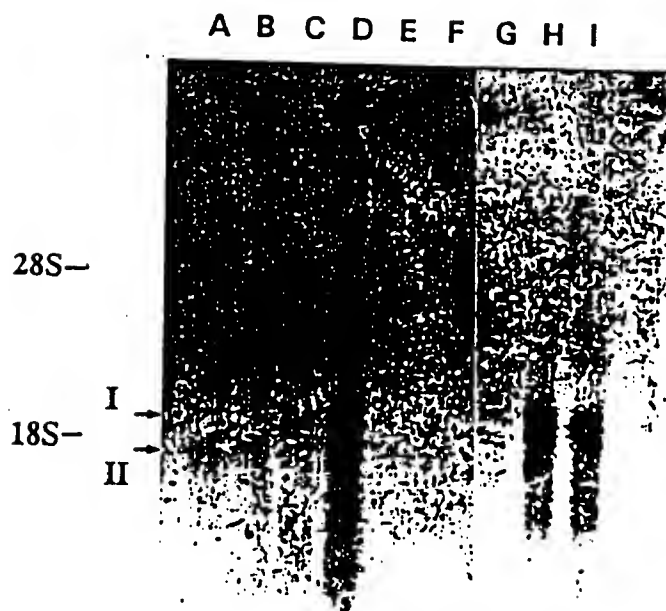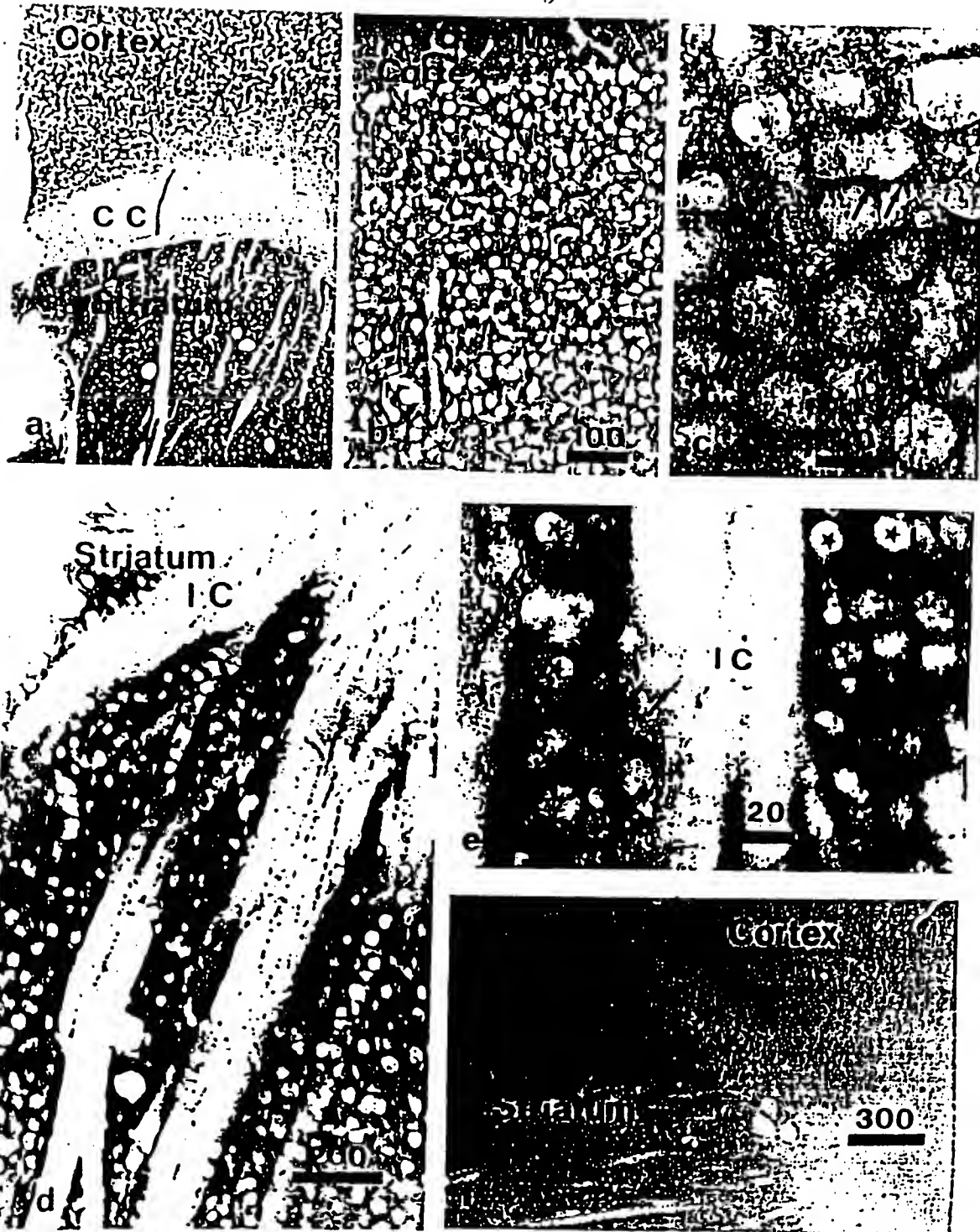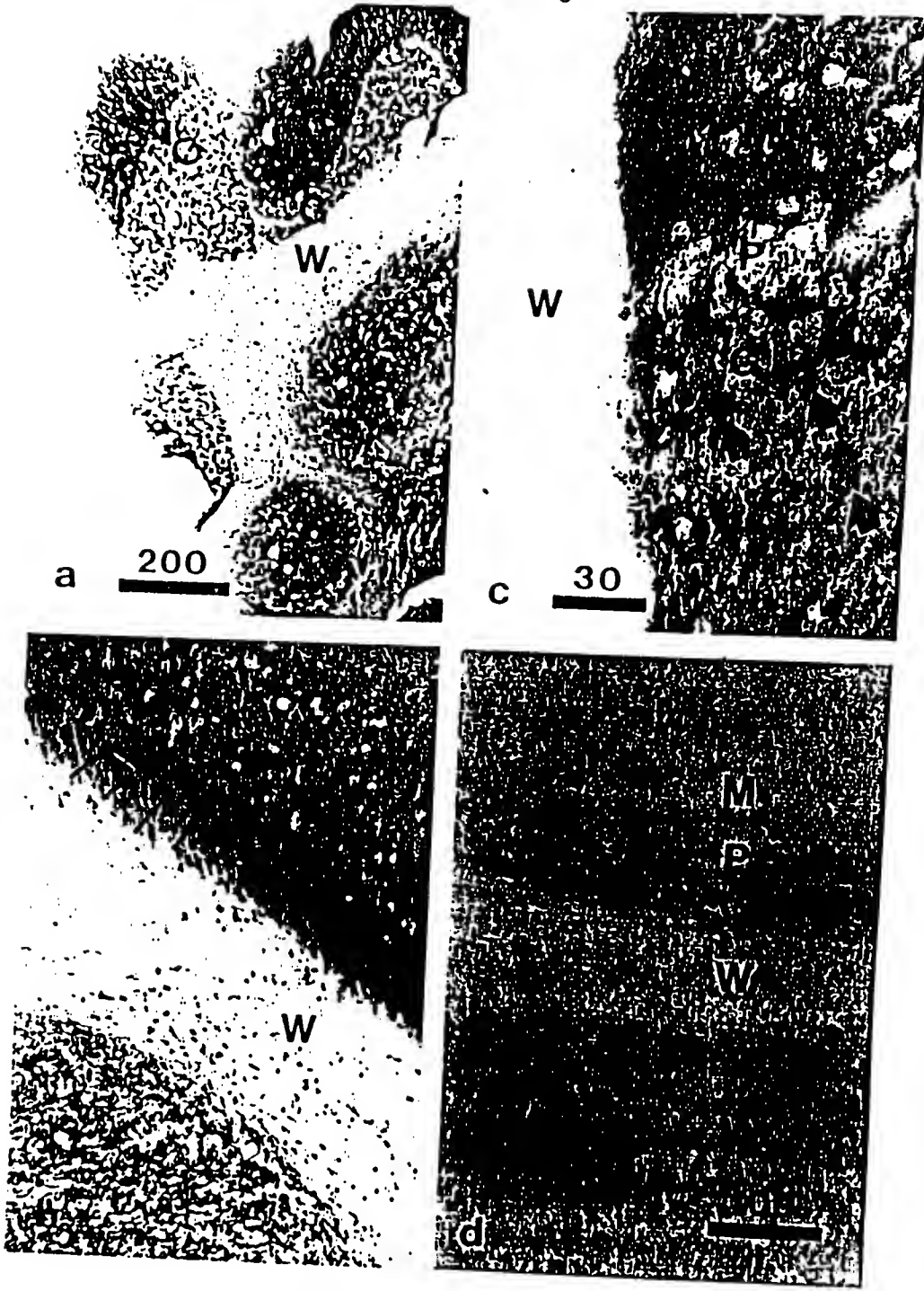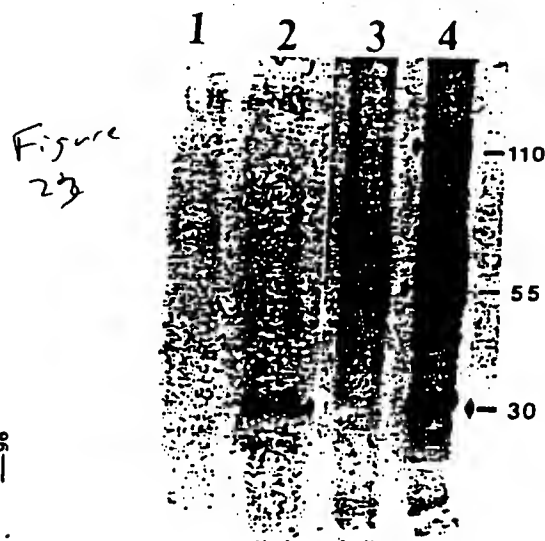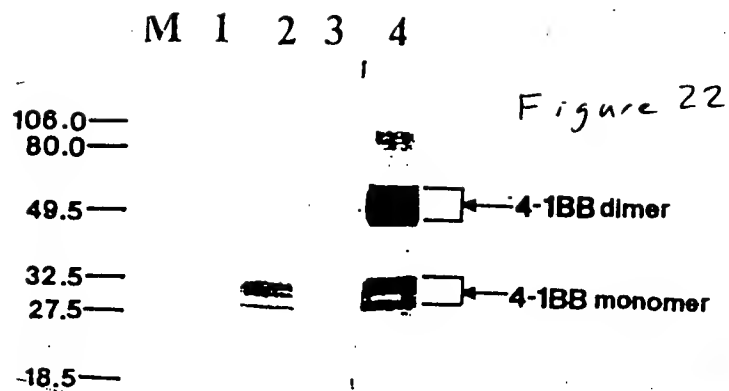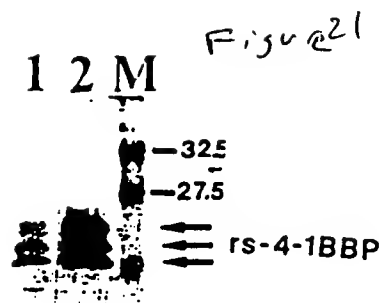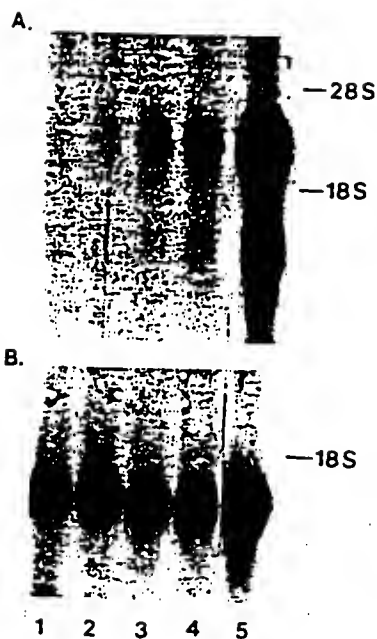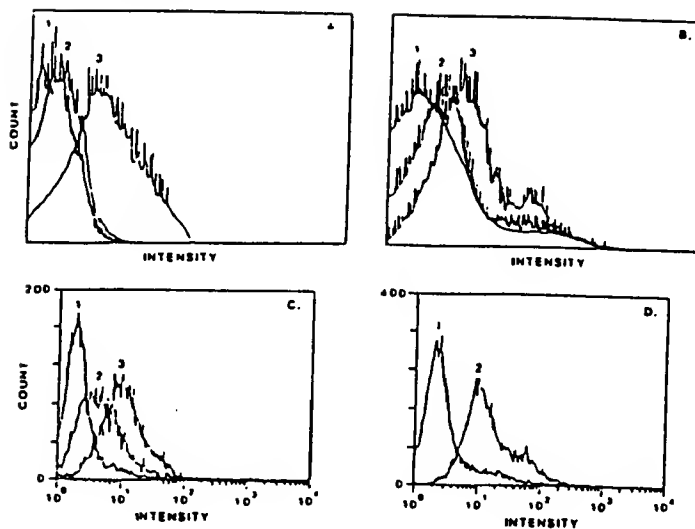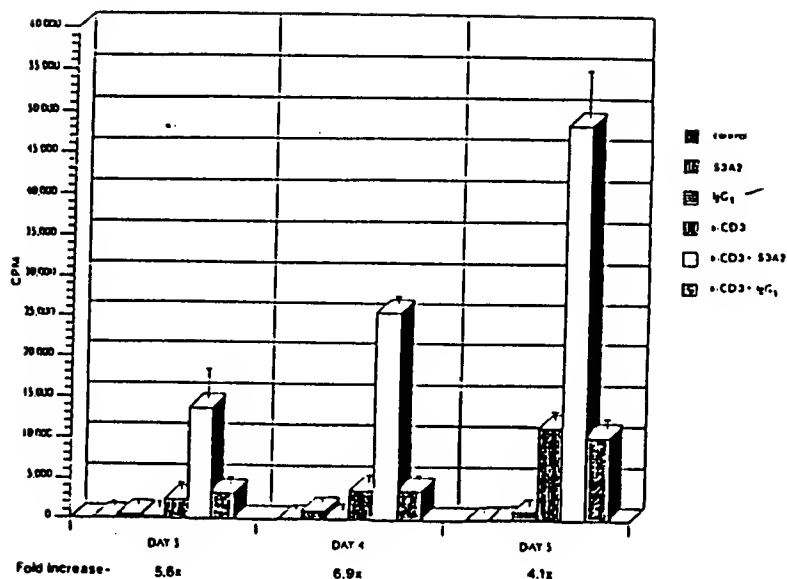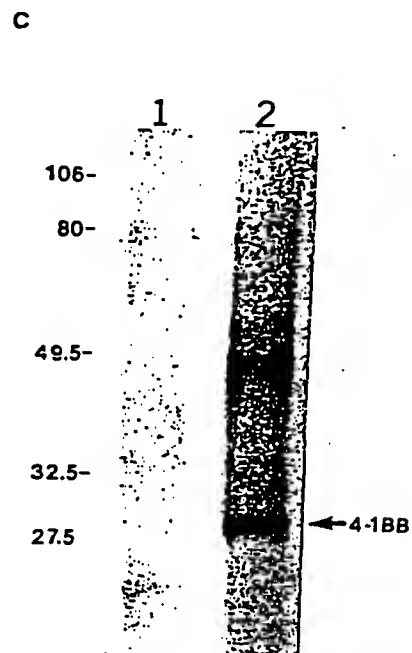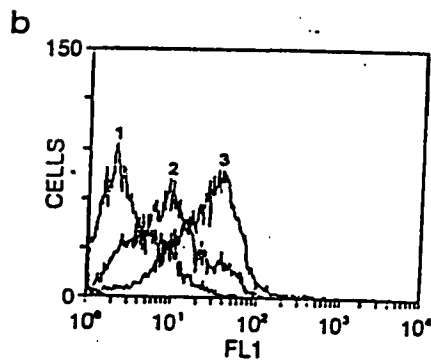
Figure 29

Figure 30

Figure 31

Figure 32

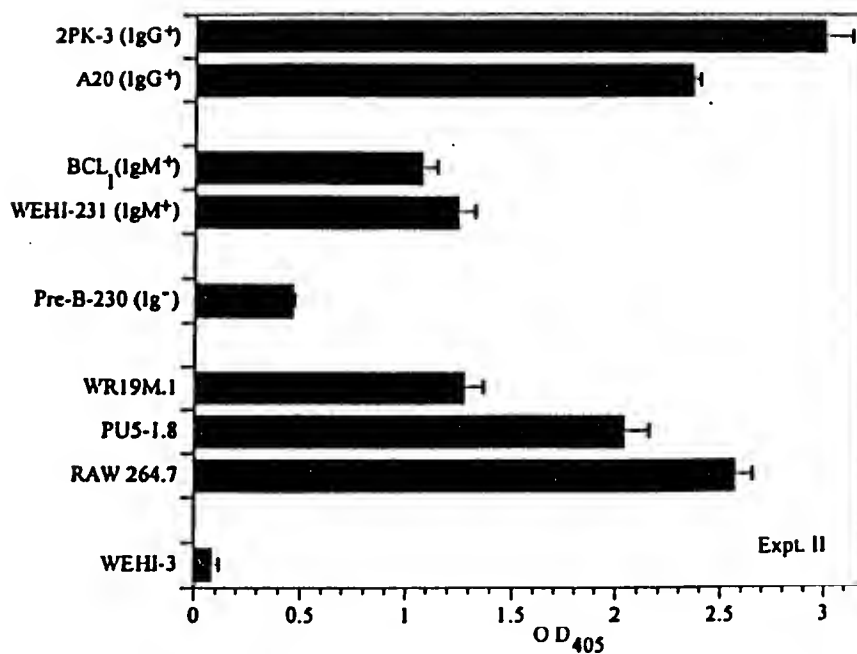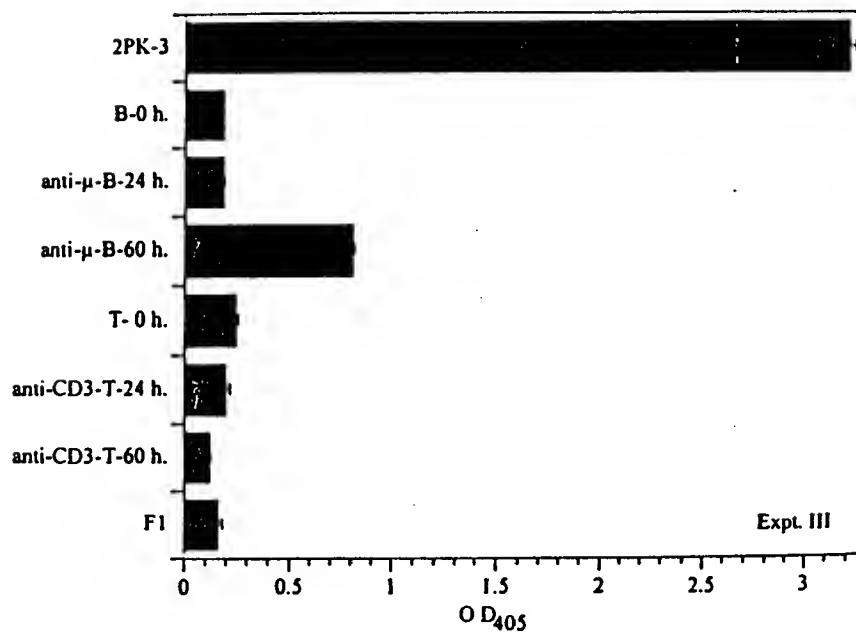

Figure 33

Figure 34a

Figure 34b

Figure 34c

	2PK-3
	B-0 h.
	anti-μ-B-24 h.
	anti-μ-B-60 h.
	T- 0 h.
	anti-CD3-T-24 h.
	anti-CD3-T-60 h.
	F1

Expt. III

O D$_{405}$

Figure 35a

A20   + 4-1BB-AP —■—
D10.G4 + 4-1BB-AP —□—

O D$_{405}$

4-1BB-AP (µg/ml)

Figure 35b

A20 + rs4-1BB + 4-1BB-AP

A20 + 4-1BB-AP

0   0.05   0.1   0.15   0.2   0.25   0.3   0.35   0.4   0.45

OD_{405}

Figure 36

C

C + SF21-4-1BB

C + SF21-wt

anti-μ (10 μg/ml)

anti-μ (10 μg/ml) + SF21-4-1BB

anti-μ (10 μg/ml) + SF21-wt

anti-μ (1.0 μg/ml)

anti-μ (1.0 μg/ml) + SF21-4-1BB

anti-μ (1.0 μg/ml) + SF21-wt

anti-μ (0.1 μg/ml)

anti-μ (0.1 μg/ml) + SF21-4-1BB

anti-μ (0.1 μg/ml) + SF21-wt

0   5   10   15   20   25   30   35   40   45

$cpm \times 10^{-3}$